binding partner. Control reaction mixtures are incubated without the test compound or with a non-active control compound. The formation of any complexes between the GLUTX moiety and the binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of GLUTX and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal GLUTX protein may also be compared to complex formation within reaction mixtures containing the test compound and a mutant GLUTX. This comparison may be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal GLUTX.

The assay for compounds that interfere with the interaction of the GLUTX and a binding partner can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the GLUTX protein, polypeptide, peptide, or fusion protein, or the binding partner onto a solid phase and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction by competition can be identified by conducting the reaction in the presence of the test substance; *i.e.*, by adding the test substance to the reaction mixture prior to or simultaneously with the GLUTX moiety and interactive binding partner. Alternatively, test compounds that disrupt preformed complexes, *e.g.*, compounds with higher binding constants

that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, either the GLUTX moiety or the interactive binding partner, is anchored onto a solid surface, while the non-anchored species is labeled, either directly or indirectly. In practice, microtiter plates are conveniently utilized. The anchored species may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished simply by coating the solid surface with a solution of GLUTX (or a domain thereof) or binding partner and drying. Alternatively, an immobilized antibody specific for the species to be anchored may be used to anchor the species to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface, *e.g.*, using a directly or indirectly labeled antibody specific for the initially non-immobilized species. Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes can be

detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected, *e.g.*, using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds which inhibit complex or which disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a preformed complex of the GLUTX moiety and the interactive binding partner is prepared in which either the GLUTX or its binding partners is labeled, but the signal generated by the label is quenched due to formation of the complex (see, *e.g.*, U.S. Patent No. 4,109,496 by Rubenstein which utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances which disrupt GLUTX/intracellular binding partner interaction can be identified.

In a particular embodiment, a GLUTX fusion can be prepared for immobilization. For example, the GLUTX or a peptide fragment thereof can be fused to a glutathione-S-transferase (GST) gene using a fusion vector, such as pGEX-5X-1, in such a manner that its binding activity is maintained in the resulting fusion protein. The interactive binding partner can be purified and used to raise a monoclonal antibody, using methods routinely practiced in